DOI: 10.5350/BTDMJB201107402

Advanced Glycation End–Products and Advanced Oxidation Protein Products in Patients with Insulin Dependent Diabetes Mellitus and First Degree Relatives

Alev Kural[1], Aysun Toker[2], Hatice Seval[1], Yasemin Döventaş[1], Filiz Basınoğlu[1], Macit Koldaş[1], Zuhal A. Sağlam[3]

[1]*Haseki Training and Research Hospital Biochemistry Laboratory, İstanbul*
[2]*Yoncalı Physical Therapy and Rehabilitation Hospital, Kütahya*
[3]*Haseki Training and Research Hospital Internal Medicine Clinic, İstanbul*

ÖZET

İnsüline bağımlı diyabetik hastalarda ve 1. Derece akrabalarında ileri glikozillenme ve ileri okside protein ürünleri

Amaç: Hiperglisemi düzeyi ve süresi ile diyabet komplikasyonlarının ilişkisi hakkında genel literatür bilgilerimiz tatmin edici düzeyde değildir. Bu çalışmamızda, oksidatif stres belirteçlerinin insüline bağımlı diyabetik hastalarda ve akrabalarında seviyelerinin belirlenmesi amaçlandı.

Gereç ve Yöntem: Çalışmamıza, 45 tip 1 diyabetes mellitus (DM) hastası ve bu hastaların 24 yakını dahil edildi. Plazma ileri okside protein ürünleri (AOPP), serum glikoz, üre, kreatinin, total kolesterol, trigliserid, HDL-kolesterol, total protein, albumin, ileri glikozillenme ürünü (AGE), ve HbA1c düzeyi ölçümleri uygun metolarla ölçüldü.

Bulgular: AOPP ve AGE düzeyleri komplikasyonlu veya komplikasyonsuz diyabetik hastalarda, diyabetik olmayan birinci derece akrabalara göre hafif yüksek bulundu; ancak istatistiksel olarak anlamlı değildi ($p<0.05$).

Sonuç: DM'lu hastalar ile diyabetik olmayan 1. derece akraba grupları arasında çalışılan parametreler karşılaştırıldığında glikoz ile AGE arasında ($r=0.25$ $p<0.05$) zayıf, trigliserid ile AOPP arasında orta derecede korelasyon bulundu ($p<0.05$, $r=0.587$).

Anahtar kelimeler: Tip I Diyabetes Mellitus, İleri glikasyon son ürünleri, AOPP, Diyabet komplikasyonları, 1.derece akrabalar

ABSTRACT

Advanced glycation end–products and advanced oxidation protein products in patients with insulin dependent diabetes mellitus and first degree relatives

Objective: There is no comprehensive information on the relationship of diabetic complications and the duration and severity of hyperglycemia. In this study, we aimed to determine oxidative stress markers in patients with insulin dependent diabetes mellitus and their first degree relatives.

Materials and Methods: 45 patients with Type I diabetes mellitus and 24 relatives of these patients were included in our study. Plasma advanced oxide protein products (AOPP), serum glucose, urea, creatinine, cholesterol, triglyceride, HDL-cholesterol, total protein, albumin, advanced glication end products (AGE) and HbA1c measurements were measured by appropriate methods.

Results: AOPP and AGE levels were found to be slightly elevated in diabetic patients with or without complications compared to the levels of first degree relatives however the difference was not statistically significant.

Conclusion: When we had compared the parameteres in two groups (insulin dependent diabetic patients and non diabetic parents or siblings), we found weak relation between glucose and AGE ($r= 0.25$, $p<0.05$), and moderate relation between triglyceride and AOPP levels ($p<0.05$, $r= 0.587$).

Key words: Type I Diabetes Mellitus, advanced glycation end products, advanced oxidation protein products, diabetes complications, first degree relatives

Bakırköy Tıp Dergisi 2011;7:130-135

INTRODUCTION

Type I diabetes mellitus (DM) is an immune-mediated disease and an organ specific autoimmune disease (1). The pathogenetic link has many varietes between hyperglycemia and the complications of diabetes. Oxidative stress has been suggested to play a primary role in this process (2). Chronic hyperglicemia in diabetes induces the oxidation and glycation of many biologically important molecules. Glycation and oxidation processes are widely called as glycooxidation. Glycooxidation proteins products leads to lipids, DNA and even membrane and endothelium damage (3). All these structural changes cause abnormality in functions of macromolecules and/

Yazışma adresi / *Address reprint requests to:* Alev Kural
Haseki Training and Research Hospital, Biochemistry Laboratory, İstanbul
Telefon / *Phone:* +90-212-529-4400/1408
Elektronik posta adresi / *E-mail address:* alevkural@hotmail.com
Geliş tarihi / *Date of receipt:* 4 Temmuz 2011 / July 4, 2011
Kabul tarihi / *Date of acceptance:* 26 Eylül 2011 / September 26, 2011

or accumulation of them in biological systems. It is believed that glycooxidation products have a basic role in development of vascular complications in diabetes (4,5).

Indirect methods that measure secondary products of oxidative modified molecules, are more practical than direct complex methods that measure directly oxidative stres. The structural similarity of advanced oxidation protein products (AOPP) and AGE proteins lead to similar biological activity at induction of proinflammatory cytokines and adhesive molecules. Additionally, accumulation of AGE and AOPP in biological systems causes damages in membranes and endothel and it is important at long term diabetic complications. All of the high and low molecular weight AOPP molecules present in plasma has been agregated in oxidized or monomeric form.

AOPP is well indicator for oxidative damage grade, because albumin is the most powerful antioxidant plasma proteins and AOPP is the end product (6).

Reactive oxygen species (ROS), lead to formation of oxide amino acid via a direct effect on proteins. The effect of reactive carbonyl compounds (RCO) which result from auto-oxidation of carbonhydrates and lipids by indirect pathway turn into AGE and advanced lipooxidation end products (ALE). AGE is known as N-carboxymethyl-lysine and pentosidine. This process keeps low intensity under physiological states. Effects of this type on soluble proteins are normally small and limited to the percentage of protein molecules actually glycated, which, in turn, depends on the half-life of the protein. The most of the primary effects of AGE leading to the complications of diabetes and aging are due to AGE formation on long-lived proteins (7).

ROS cause protein aggregation and fragmantation by forming ditirozin which is generated from direct oxidation of tirozin aminoacid. The products produced by these mutual bonds are totally called AOPP (8). Witko-Sarsat et al. described the presence in the plasma of haemodialysed patients high levels of oxidized proteins , designated as AOPP (9). There are two UV-visible peaks of absorbance at 340 nm corresponding to a molecular mass of 60 (low molecular weight) and 600 kDa (high molecular weight) of AOPP. Neutrophils which contain the most important source of chlorined oxidants due to their high content in myeloperoxidase, may be involved in plasma AOPP formation (10). AOPP were defined a novel marker of oxidative damage and considered as confidental markers to estimate the degree of oxidant-mediated protein damage (9,11).

The relation between AOPP and monocytes suggest that AOPP is not only a marker of oxidative stress but also a marker of inflammatory response because of induction of proinflammatory cytokines and adhesive molecules due to presence of AOPP (9,12,13,14). In our study, by measuring the plasma AOPP and serum AGE levels as oxidative stress markers, we aimed to determine whether there is a relationship between oxidative stress markers and diabetic complications or not, and to determine oxidative stress marker levels in first degree relatives of type I diabetics.

MATERIALS AND METHODS

A total of 45 patients with type I DM and 24 nondiabetic relatives of these patients (12 parents , 12 siblings) were included in our study. The study protocol was approved by the institutional ethics committee. All participants gave informed consent recruitment. Demographic characteristics of the diabetic patients and their nondiabetic relatives are presented in Table 1. The relative's group was chosen amongst those who hadn't have diabetes or any other systemic disease. None of the subjects was taking any drugs which may effect lipid metabolism and oxidative status at the time of the study.

From all subjects, venous blood samples were taken after an overnight (10 hours) fasting and samples were separated and stored frozen at −80°C until analysis. The levels of total cholesterol, triacylglycerol (TAG), glucose, HDL-cholesterol, total protein, albumin, urea, creatinine

Table 1: The demographic characteristics of the Type 1 diabetic patient group and nondiabetic relatives

	Type 1 Diabetic Patients (n= 45)	Nondiabetic relatives (n= 24)	p
Age (years) (mean±SD)	29.8±9.2	36.04±11.7	>0.05
Gender (F/M)	33/12	18/6	>0.05
Smoking (yes/no)	14/31	11/13	>0.05
Complication (yes/no)	15/30		

Table 2: The results of biochemical analytes in Type 1 diabetic patients and their nondiabetic relatives

Parameters	Type 1 Diabetic Patients (n= 45)	Nondiabetic relatives (n= 24)
Glucose (mg/dl)	216±109.07[2]	102.5±34.2
Total cholesterol (mg/dl)	167±38.1	176.4±40
Triacylglycerol (mg/dl)	100.9±45[3]	98.2±60.9
HDL-cholesterol (mg/dl)	52.8±13.9	47±11
Total Protein (g/dl)	7.15±0.65	7.27±0.48
Albumin (g/dl)	4.38±0.38	4.5±0.24
Creatinine (mg/dl)	0.9±0.9	0.82±0.15
HbA1C (%)	8.17±1.5[1]	5.01±1.17
AOPP/Alb (μmol/L/g)	8.4±3.5[3]	7.94±3.38
AGE /Total Prot (AU/g protein)	18.19±5.85[2]	15.7±6.36

AGE: Advanced glycation end-products, AOPP: Advanced oxidation protein products

were determined by enzymatic methods using Olympus AU 2700 (Clare, Ireland) autoanalyzer. HbA1c levels were detected by immunoassay method in the same autoanalyzer. LDL-cholesterol was calculated by the formula of Friedewald et al (15). None of the patients had TAG> 400 mg/dl.

Serum AGE levels were determined by spectrofluorimetrically (emission 440 nm, excitation 350 nm) at Biorad Fluorometer (San Diego, CA) analyzer according to the method described by Kalousova (16). All samples were diluted at 1:50 ratio with PBS (pH 7.4). Results are expressed in arbitrary units (AU)/g protein.

Plasma AOPP levels were determined by spectrophotometric method. Plasma samples were diluted at 1:50 ratio with PBS (pH 7.4). Cloramin T (sigma) was used at 0-100 μmol/l concentration range for calibration. The reagents were prepared with solution of 1.16 M KI and 20 μl absolute acetic acid. The method was adopted to Olympus AU 2700 analyzer and the measurement was performed at 340 nm. AOPP levels were assessed with cloramin T unit (μmol/L). The corrected values according to serum albumin levels were calculated (μmol/L/ g albumin) (16).

Statistical analyses were performed using SPSS 13.0 program. The results are given as mean±SD. Primarily, data were evaluated as three groups (patients with complications, patients without complications and nondiabetic relatives of these patients). Mann-Whitney U test was applied and correlation analysis was performed using Spearman test. The threshold of statistical significance was defined as $p< 0.05$.

RESULTS

The results of the serum and plasma biochemical analytes are summarized in Table 2. Mean HbA1C level was 8.2±1.2% in the patient group. Only significant difference was found between mean levels of glucose and HbA1C among diabetic patients and their relatives ($p< 0.001$).

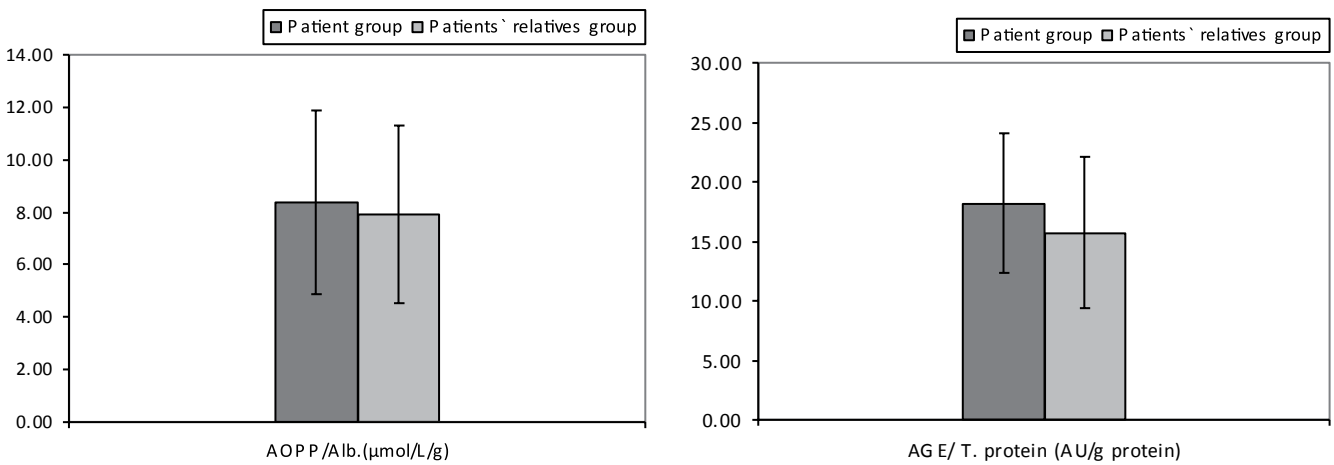

Figure 1: Concentration of mean AOPP (A) and AGE (B) levels in Tip 1 diabetic patients and nondiabetic relatives. Non significant increase compared to non diabetic relatives (p>0.05).

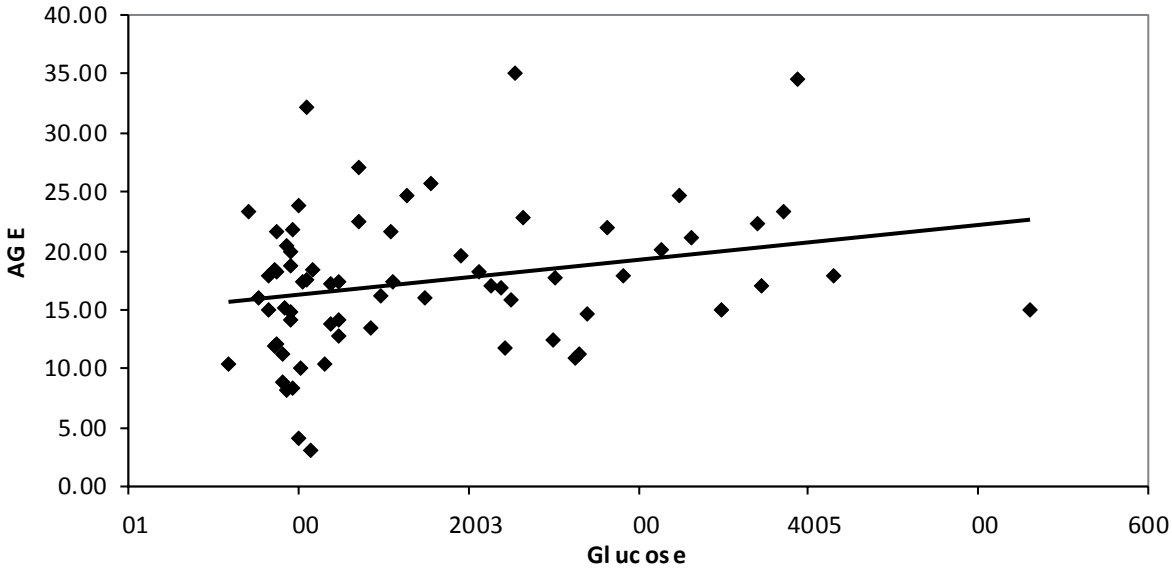

Figure 2: Correlation between AGE and glucose in diabetic patients (r = 0.241, p < 0.01).

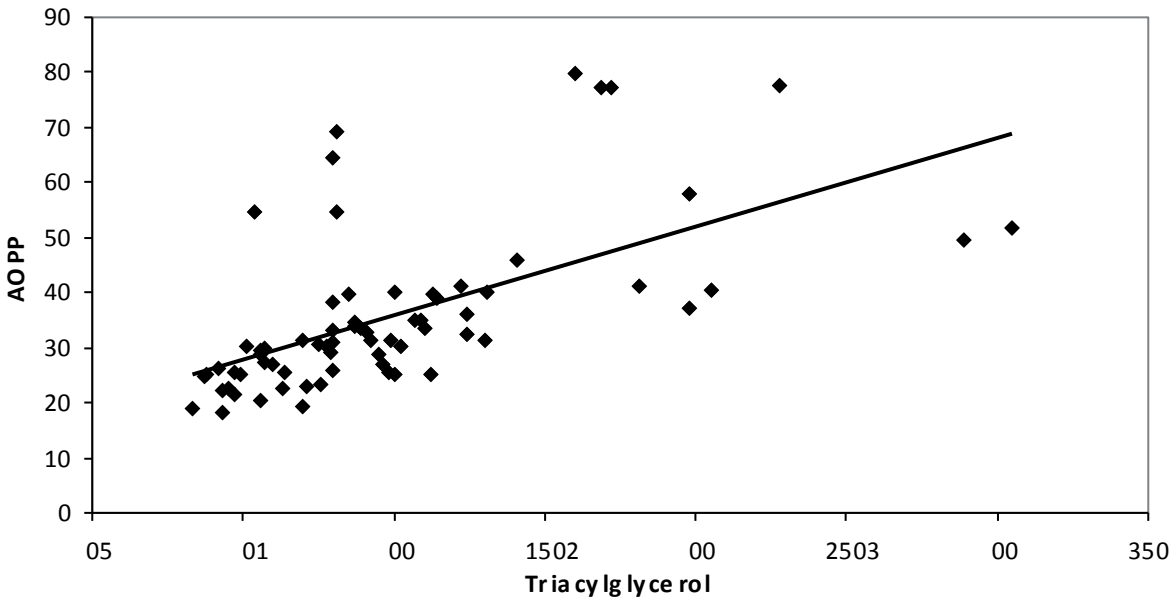

Figure 3: Correlation between AOPP and TAG levels in diabetic patients (r = 0.587, p < 0.05).

Both AGE and AOPP levels were higher in patients with complications (19.41±5.6 AU/g protein, 9.12±4.5 μmol/L/g albumin respectively) in comparison with diabetic patients without complications such as nephropathy, retinopathy, neuropathy (17.59±6.0 AU/g protein, 7.36±2.6 μmol/L/g albumin respectively). But this differences are not statistically significant (p>0.05).

In the diabetic patient group, serum AGE and plasma AOPP levels were higher than relatives but this differences are not statistically significant either (p>0.05) (Figure 1). Among type 1 diabetic patients and their relatives the significant correlation were found between serum glucose and AGE levels (Figure 2) and between plasma AOPP and serum TAG levels (Figure 3) (respectively; r= 0.241, p< 0.01, r= 0.587, p< 0.05).

DISCUSSION

Diabetes mellitus is a pathologic condition related with hyperglycemia, increased non enyzmatic glycation, oxidative stress and carbonyl stress (17,18). It has been shown that elevated concentrations of Amadori products

are associated with diabetic atherogenesis by activating vascular smooth muscle cells (19).

Hyperglycemia in diabetes can generate free radicals, hydrogen peroxide and reactive aldehydes by different molecular mechanism: the auto-oxidation of glucose, from interaction between glycated proteins (AGE) and the receptor of AGE in macrophage and messangial cells and , activation of protein kinase C which is implicated in regulation and activation of various membrane associated NAD(P)H dependent oxidases (20-22).

Some studies suggest that the determination of AGE and AOPP compounds are important at explanation of damage mechanisms in diabetic patients (9,16).

In our study we determined the relation between plasma AOPP and serum AGE levels as oxidative stress markers with diabetic complications, disease duration, and the other parameters studied in Type 1 DM patients. We also determined the relationship between oxidative stress and tendency to diabetes in first degree relatives of type I diabetics. Similarly in another study among diabetics and their relatives the significant correlation was found between TAG and AOPP levels in our study ($r= 0.587$, $p<0.05$)(16). Although Kalousova et al. did not find any significant correlation between AGE and glucose levels, in our study, there was significant slight correlation between AGE and glucose levels ($p<0.05$, $r=0.241$) (16). No significant correlation between oxidative stress markers and the parameters studied such as total cholesterol, HDL-cholesterol, LDL-cholesterol were found as some researches (3,16) Plasma AOPP and serum AGE levels in diabetic patients were higher than in nondiabetic relatives. But it was not statistically significant.

It was showed that variable oxidant, antioxidant balance may be a risk factor in pathologic conditions such as retinopathy, neuropathy, cataract and atherosclerosis (20,23).

Piwowar et al. compared AOPP levels in long term (more than 10 years) and short term (less than 5 years) Type 2 diabetic patients (3). They reported significant high levels in long term patients and they had a clinical trial about relation between gluoxidation markers and Type 1 and 2 DM (24). In our study, we did not find any correlation between plasma AOPP with serum AGE levels and diabetic complications, disease duration, kind of complication in Type 1 diabetic patients.

We conclude that there are no relation between plasma AOPP and serum AGE levels as oxidative stress markers, whether complication development or not, kind of complication such as nephropathy, retinopathy, neuropathy. Although there are limited data about how reactive carbonyl compounds make role in hyperglycemia, hyperlipidemia, and of their complications, further studies should be done about this compounds for delaying or prevention diabetic complications.

REFERENCES

1. Bach JF. Insulin-dependent diabetes mellitus as an autoimmune disease. Endocrine Rev 1994; 15: 516-542.
2. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991; 40: 405-412.
3. Piwowar A, Knapik-Kordecka M, Szczecinska J, Warwas M. Plasma glycooxidation protein products in type 2 diabetic patients with nephropaty. Diabetes Metab Res Rev 2008; 24: 549-553.
4. Abou-Seif MA, Youssef AA. Evaluation of some biochemical changes in diabetic patients. Clin Chim Acta 2004; 346: 161-170.
5. Martin-Gallan P, Carrascosa A, Gussinye M, Dominguez C. Biomarkers of diabetes associated oxidative stress and antioxidant status in young diabetic patients with or without subclinical complications. Free Radic Biol Med 2003; 34: 1563-1574.
6. Baskol G, Gumus K, Öner A, Arda H, Karaküçük S. The role of advanced oxidation protein products and total thiols in diabetic retinopathy. Eur J Ophthalmol 2008; 18: 792-798.
7. Ulrich P, Cerami A. Protein glycation, diabetes, and aging. Recent Prog Horm Res 2001; 56: 1-21.
8. Gil-del Valle L, de la C Millian L, Toledo A, Vilaro N, Tapanes R, Otero MA. Altered redox status in patients with diabetes mellitus type I. Pharmacol Res 2005; 51: 375-380.
9. Witko-Sarsat V, Friedlander M, Capeillere-Blandin C, et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int 1996; 49: 1304-1313.
10. Witko-Sarsat V, Friedlander M, Nguyen-Khoa T, et al. Advanced oxidation protein products as novel mediators of inflamation and monocyte activation in chronic renal failure. J Immunol 1998; 161: 2524-2532.
11. Alderman CJ, Shah S, Foreman JC, Chain BM, Katz DR. The role of advanced oxidation protein products in regulation of dendritic cell function. Free Radic Biol Med 2002; 32: 377-385.
12. Descamps-Latscha B, Witko-Sarsat V. Importance of oxidatively modified proteins in chronic renal failure. Kidney Int Suppl 2001; 78: 108-113.
13. Tsukahara H, Sekine K, Uchiyama M, et al. Formation of advanced glycosylation end products and oxidative stress in young patients with type 1 diabetes. Pediatr Res 2003; 54: 419-424.
14. Selmeci L, Szekely M, Soos P, et al. Human blood pasma advanced oxidation protein products (AOPP) correlates with fibrinogen levels. Free Radic Res 2006; 40: 952-958.
15. Friedewald WT, Levry RI, Frederickson DS. Estimation of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-502.

16. Kalousova M, Skrha J, Zima T. Advanced glycation end-products and advanced oxidation protein products in patients with diabetes mellitus. Physiol Res 2002; 51: 597-604.

17. Vlassara H. Recent progress in advanced glycation end products and diabetic complications. Diabetes 1997; 46: 19-25.

18. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes 1999; 48: 1-9.

19. Hattori Y, Suzuki M, Hattori S, Kasai K. Vascular smooth muscle cell activation by glycated albumin (Amadori adducts). Hypertension 2002; 39: 22-28.

20. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001; 414: 813-820.

21. Yan SD, Schmidt AM, Anderson GM, et al. Enhanced cellular oxidant stres by the interaction of advanced glycation end products with their receptor/binding proteins. J Biol Chem 1994; 269: 9889-9897.

22. Lander HM, Tauras JM, Ogiste JS, Hori O, Moss RA, Schmidt AM. Activation of the receptor for advanced glycation end products trigger a p21(ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stres. J Biol Chem 1997; 272: 17810-17814.

23. Retinopathy and nephropathy in patients with type I diabetes four years after a trial of intensive therapy. The Diabetes Control and Complications Trial, Epidemiology of Diabetes Interventions and Complications Research Group. N Engl J Med 2000; 342: 381-389.

24. Piwowar A, Knapik-Kordecka M, Warwas M. AOPP and its relation with selected markers of oxidative /antioxidative system in type 2 diabetes mellitus. Diabetes Res Clin Pract 2007; 77: 188-192.